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EGGS OF *PALAEMON MACRODACTYLUS:* I. ATTACHMENT TO THE PLEOPODS AND FORMATION OF THE OUTER INVESTMENT COAT

WILLIAM S. FISHER AND WALLIS H. CLARK, JR.

Aquaculture Program, Animal Science Department, University of California, Davis, CA 95616

ABSTRACT

Eggs of the estuarine shrimp, *Palaemon macrodactylus,* were examined for their method of attachment during the brooding period. They were attached to each other and to setae of the maternal pleopods by an externally applied outer investment coat that is believed to originate within the pleopods. A precursor substance was stored in packets extending longitudinally along the basipodites of the female pleopods, apparently originating in specialized epithelial cells continuous with the packets. At ecdysis prior to oviposition, the packets became depleted and the attachment or adhesive material became evident externally, conforming to the external surfaces of the pleopods. This occurred in the presence and absence of mature eggs in the ovary. If mature eggs were present, they were soon (within one-half day) extruded into the incubation chamber, coated with the adhesive material, and attached. Both fertilized and unfertilized eggs attached in the same manner, although unfertilized eggs were either shed or removed by the female within a few days. Eggs from pleopod-excised females did not attach, had no outer investment coat, and deteriorated rapidly. This attachment mechanism differs from those described for other decapods.

INTRODUCTION

In certain decapod crustaceans, the females incubate their embryos on pleopods (swimmerettes) on the abdomen until hatching. During this period, the embryo's investment coats (egg coats, egg envelopes) protect it from physical and chemical stresses and maintain the internal milieu. The outer investment coat, due to its immediate exposure to the aquatic environment, is of primary importance in this role. The outer coat has also been associated with the attachment of eggs to the maternal pleopods, selective permeability (Yonge, 1937), and osmotic hatching (Davis, 1965), and it may serve as a substratum for aquatic microorganisms (Johnson *et al.,* 1971). Several investigators, often using different species of brooding decapods, have presented conflicting theories concerning the origin and formation of this important protective layer. It is the intent of this study to examine the outer investment coat of the estuarine shrimp *Palaemon macrodactylus,* and compare its egg attachment process with that described for other species. The word "egg" is often used in the general sense to identify either unfertilized eggs, fertilized eggs, or both. When necessary, these will be specifically designated as "fertilized" or "unfertilized." Fertilized eggs are also appropriately termed "embryos."

An early theory of attachment (Yonge, 1937) was based on work with the macruran *Homarus vulgaris,* which was found to have glands in the female pleopods that secreted a material to coat the newly extruded eggs. This material simultaneously formed the outer investment coat and attached the eggs to the pleopodal setae. Yonge's hypothesis explained Andrews' (1906) observations, which reported the

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secretion of a mucous substance into the incubation chamber of crayfish just prior to egg extrusion. In contrast, Burkenroad (1947) felt the outer coat from eggs of the caridean *Palaemonetes vulgaris*, was formed by the newly extruded eggs upon contact with a medium such as sea water. He believed the outer coat was inherently capable of fusing with other eggs or maternal setae for attachment, but in some cases required an "intensifier substance" such as the material released by the pleopodal glands of the macrurans.

Another contrasting theory was presented by Cheung (1966) who found three embryonic coats for the brachyuran *Carcinus maenas*. All were formed by the ovum as a consequence of fertilization. Moreover, Cheung found no pleopodal "cement glands" associated with oviposition, a result that conflicted with reports on other decapods (Andrews, 1906; Yonge, 1937; Lloyd and Yonge, 1940; Stephens, 1952). The absence of pleopodal glands coupled with exclusive egg-to-seta attachment also contradicted the self-fusibility theory of Burkenroad (1947). Yet recent ultrastructural evidence from *C. maenas* (Goudeau and Lachaise, 1980) and from the spider crab *Libinia emarginata*, (Hinsch, 1971) supports Cheung's concept that the outer investment coat was formed from the vitelline envelope.

Cheung cited Hoglund (1943) and Jefferies (1964) to support his claim that carideans also required fertilization for egg attachment. Both investigators found that unfertilized eggs of shrimp would be extruded into the brood chamber, but they would not have an outer investment coat and would either not attach or would be shed within hours. Not cited by Cheung (1966), however, was the more common occurrence described by Jefferies (1964) of unfertilized eggs attaching for up to five days. Burkenroad (1947) also found that unfertilized *P. vulgaris* eggs became attached but, rather than being shed, were removed by the female within a few days. Unfertilized eggs remained attached when the female pereiopods were amputated, thus negating fertilization as a prerequisite for attachment. The ability to attach unfertilized eggs has also been noted for another Palaemonidae, *Macrobrachium rosenbergii* (Bardach *et al.*, 1972), and is described in this paper for an estuarine shrimp, *Palaemon macrodactylus*.

The reproductive pattern of *P. macrodactylus* is similar to that described for *M. rosenbergii* (Bardach *et al.*, 1972) and *Leander squilla* (Hoglund, 1943). Within hours after the female molts, the male deposits spermatophores externally on her ventral thorax. Eggs are extruded within 2–3 hours of mating, fertilized, and attached to pleopodal setae in the incubation chamber. *P. macrodactylus* was particularly well-suited for these studies because of its capacity to extrude eggs nearly every two weeks from April to October.

Materials and Methods

Specimens of *P. macrodactylus* were collected from the Petaluma River in northern California and maintained from April to October in individual compartments in a well-aerated, closed circulating system at the University of California, Davis campus. Animals were kept at 21 to 25°C and 10 to 20 g/l salinity and were fed *Artemia salina* or frozen bay shrimp. Molting and egg extrusion were monitored daily. Animals were mated by placing a male in a female's compartment a few days before her expected molt. Egg extrusion and attachment took place for both mated and unmated females.

Eggs were obtained for microscopy directly from the ovary (unextruded), as attached unfertilized eggs (from unmated females), as attached fertilized eggs (from mated females), and as extruded but unattached eggs collected by excising the first

or second pair of maternal pleopods before extrusion. This last procedure caused the eggs to fall directly from the oviduct to the floor of the aquarium. Samples of eggs and excised pleopods from animals in different stages of the reproductive cycle were fixed in 2% glutaraldehyde in a standard 0.1 *M* phosphate buffer at pH 7.3. Samples were osmified (0.1% OsO_4), embedded in a low viscosity epoxy resin (Spurr, 1969) and sectioned on a Porter Blum MT-2 ultramicrotome. Thick sections (0.5 μm) were stained 10–15 seconds with borate buffered toluidine blue (Dewel and Clark, 1972). Fixed tissues were also embedded in butoxyethanol/glycol methacrylate medium (Scientific Chemical Co., Huntington Beach, CA.) and stained with periodic acid-Schiff's (PAS) reagent (Lillie, 1965). Samples were viewed with compound light microscopy.

Results

During this study, female *P. macrodactylus* held in the system molted every 16.3 (±3.5 days), and eggs were extruded and attached to the pleopods after 76.5% of these molts, usually within 1 day. Unfertilized eggs were lost or removed from the pleopods after 1 to 3 days, while embryos remained attached for an average 14.3 (±0.8) day incubation period. Eggs teased from the ovary appeared to have a material on their surfaces that would form a coagulum on contact with water from the holding system. This coagulation caused the eggs to adhere to each other in a clump, but the clump could be teased apart easily with forceps.

During oviposition, the female stood upright and the eggs moved into a chamber formed by the pleopods and the lateral epimera (pleura) on the underside of her abdomen (Fig. 1). Eggs were attached to each other (Fig. 2) and to pleopodal setae by a connecting or adhesive material that formed the outer investment coat (Fig. 3). This occurred for both fertilized and unfertilized eggs. At sites of attachment, the adhesive material took the shape of a flattened strand ("connectant," Fig. 4) or a twisted stalk ("funiculus," Fig. 5).

Cross sections of mature eggs taken from the ovary had a single investment coat (the vitelline envelope) that appeared separated from the egg, probably caused during fixation (Fig. 6). Naturally spawned eggs that were attached to pleopods had an additional (outer) investment coat (Fig. 7). This external coat was PAS-positive, was of variable thickness, and connected the eggs to each other and to the pleopods; as such, it was the adhesive material previously described. Eggs not allowed to attach (from pleopod-excised females) resembled ovarian eggs in lacking the outer coat (Fig. 8). Also like ovarian eggs, these unattached eggs adhered to one another in water and could be teased apart easily. Such eggs deteriorated within 2 days; only the vitelline envelope remained, devoid of ooplasm and appearing as a "ghost capsule" (Fig. 9). This deterioration did not occur when normally attached eggs, fertile or infertile, were detached from the maternal pleopods.

Pleopods were examined to determine the origin of the adhesive material that formed the outer coat of the eggs. Moving distally, each pleopod (Fig. 10) is joined to the abdominal sternite by the precoxae and consists of a joint called the coxapodite, a long basipidite, an endopodite, and an exopodite. The posterior face of the basipodite is concave and, starting at the proximal end, has a central channel extending one-third to one-half the length of the basipodite (Fig. 11). A cross-section of the basipodite from a female with attached eggs (Fig. 12) shows the adhesive material intimately associated with the recesses of the channel. The adhesive material conforms to the external surface of the pleopod and wraps around pleopodal setae and the extruded eggs, thereby forming the outer investment coat. Figure 13 shows

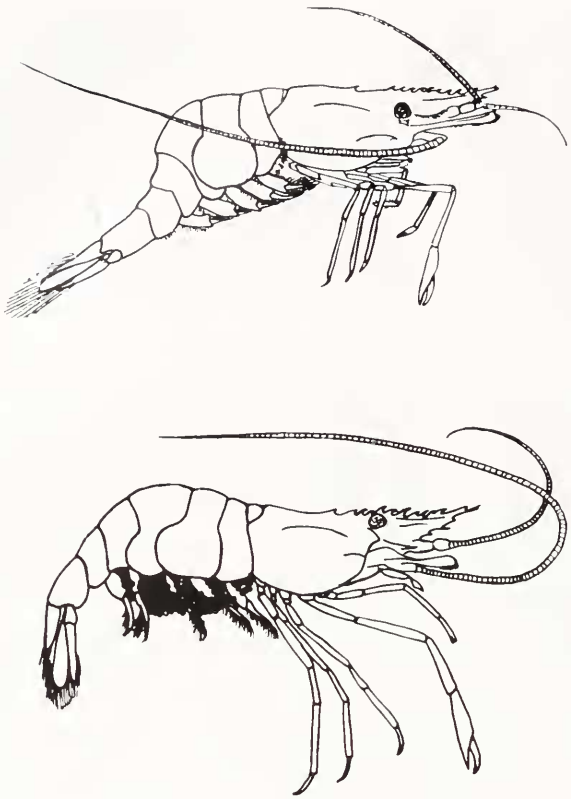

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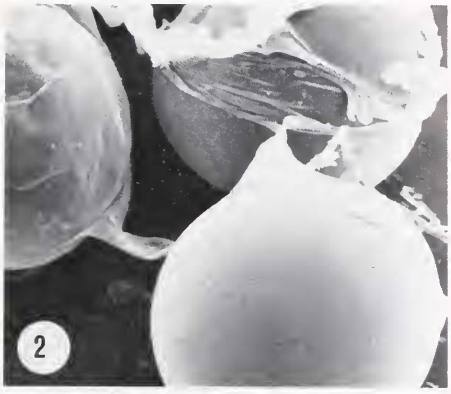
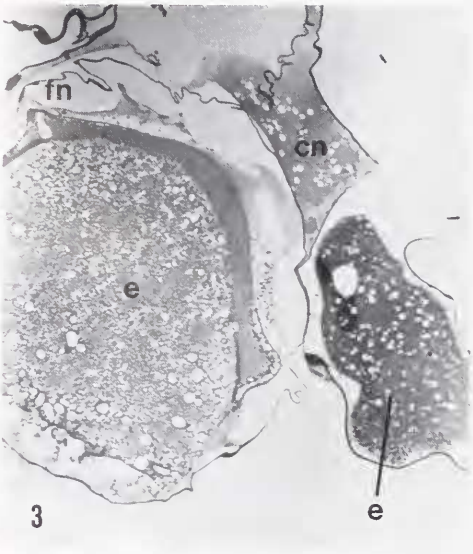
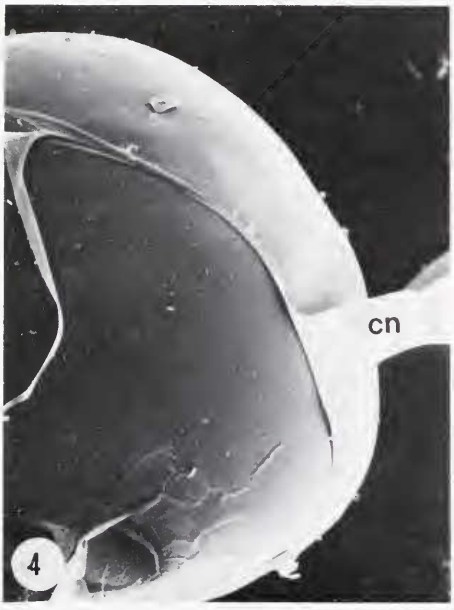
FIGURE 1. Top; illustration of a female *Palaemon macrodactylus,* extruding eggs from the oviduct into a chamber formed by the lateral plates (epimura) of the abdomen and the anterior extensions of the pleopods. Bottom; the extruded eggs are attached to the setae of the pleopods for a two-week brooding period.

this material enveloping a well-developed embryo. Adhesive material appeared at ecdysis prior to egg extrusion, but its presence was not necessarily related to extrusion since it appeared at ecdysis even when no ova were in the ovary.

Although the adhesive material was externally associated with the central channel of the basipodite, no ducts or direct portals from the interior of the pleopod were observed along the length of the channel. There were, however, long tubules or packets that contained slightly PAS-positive material. These packets (designated mucilage packets) appeared continuous with specialized epithelial cells (mucilage cells) found only in the coxapodite (Figs. 14, 15). The packets extended longitudinally into the basipodite (Fig. 16) and, proceeding distally, decreased in number until none could be found. Mucilage packets occurred in anterior, posterior, and median regions of the pleopod and, other than their proximal continuity with mucilage cells, showed no particular association with a cell or tissue type (Fig. 17). Mucilage packets were found prior to ecdysis (Fig. 18), but were absent or depleted immediately afterwards, coinciding with the appearance of adhesive material on the external surfaces of the pleopods (Fig. 19).







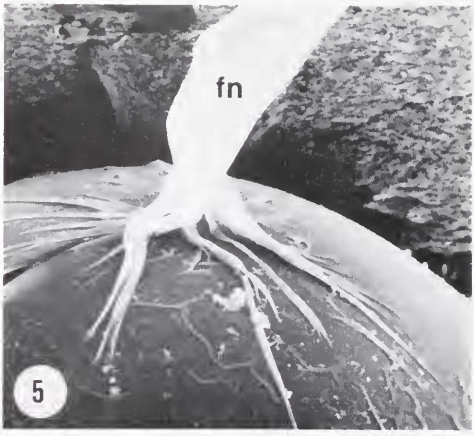


FIGURE 2. Scanning electron micrograph of eggs attached to one another by adhesive material. 80×.

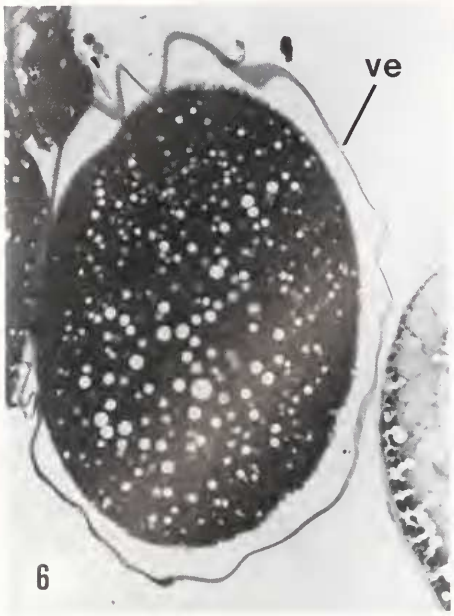
FIGURE 3. Cross-section of unfertilized eggs (e), showing adhesive material in the form of a connectant (cn) to other eggs or a funiculus (fn) to pleopodal setae. PAS-stain. 135×.

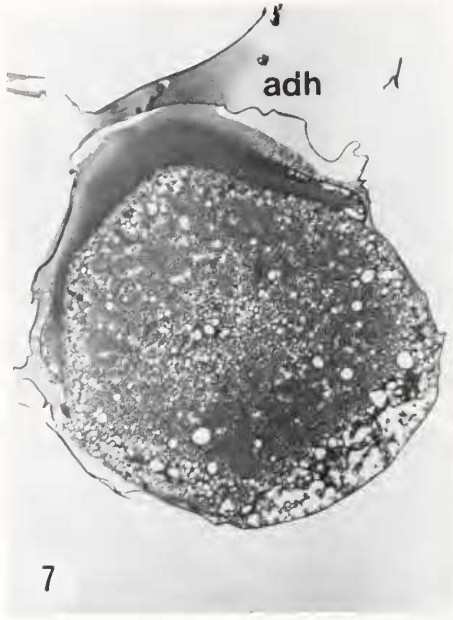
FIGURE 4. Scanning electron micrograph of adhesive material in the form of a connectant. 175×.

FIGURE 5. Scanning electron micrograph of adhesive material in the form of a funiculus. 320×.

DISCUSSION

The externally brooding caridean *Palaemon macrodactylus* exhibited a mechanism of egg attachment that differs from accounts of macruran and brachyuran egg attachment. A substance produced and stored in the female pleopods appeared to be released at molt to coat the external surfaces of the pleopods. Extruding eggs, fertilized or unfertilized, were connected to the pleopodal setae and to each other by the adhesive material, which simultaneously formed the outer investment coat of the eggs. This mechanism is unlike that suggested for *Homarus* (Yonge, 1937)





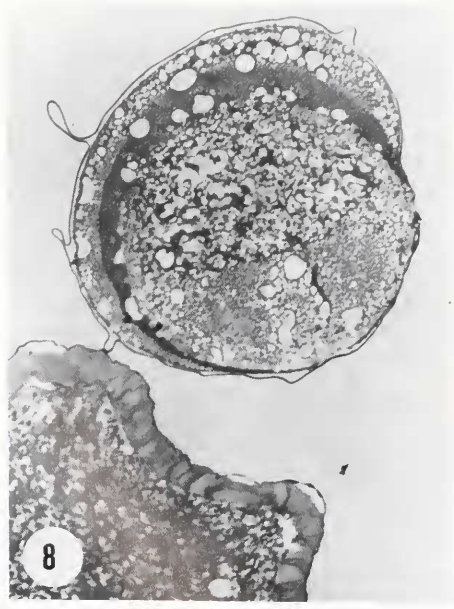
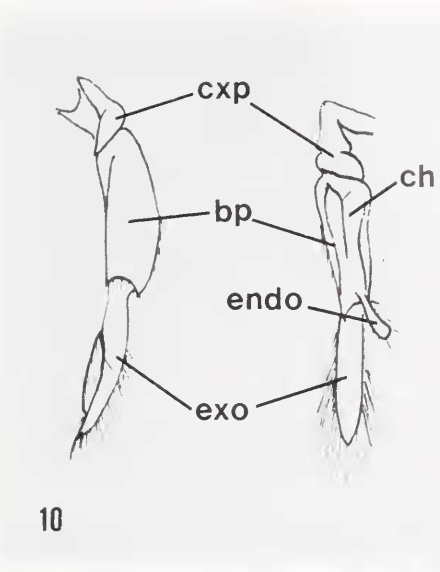


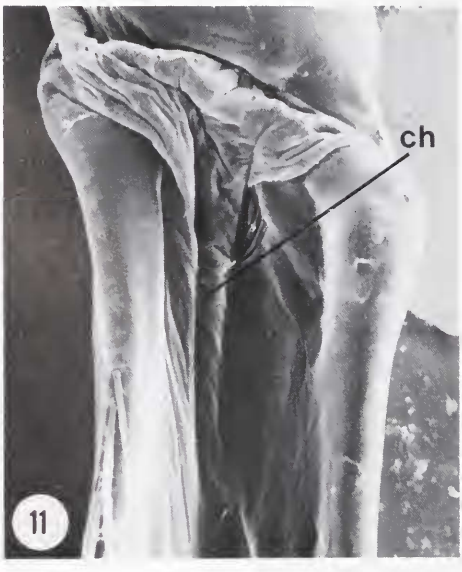
FIGURE 6. An ovarian egg showing the vitelline envelope (ve) which has been artificially elevated from the oolemma by fixation. Toluidine blue stain, 220×.

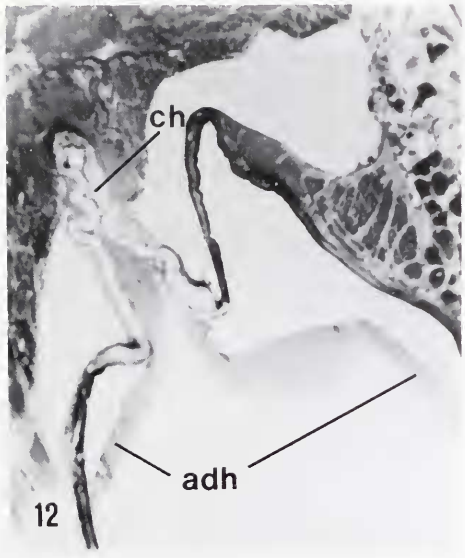
FIGURE 7. A naturally spawned, unfertilized egg detached from a maternal pleopod. The adhesive material (adh) forms the outer investment coat of the egg. PAS-stain, 180×.

FIGURE 8. Eggs from a pleopod-excised female extruded without becoming attached to maternal pleopods; they showed no sign of the adhesive material that forms the outer investment coat of naturally spawned eggs. PAS-stain, 145×.

FIGURE 9. Deterioration of eggs that were never attached (such as those in Fig. 8) after 2 days; only "ghost capsules" remain. PAS-stain, 170×.







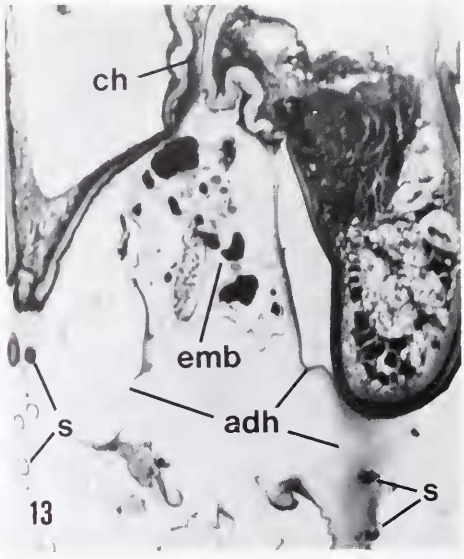
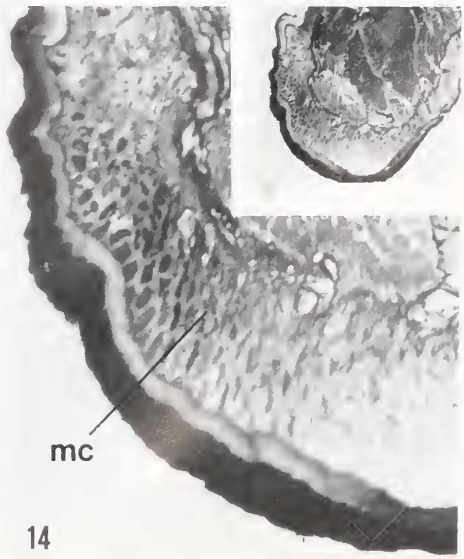


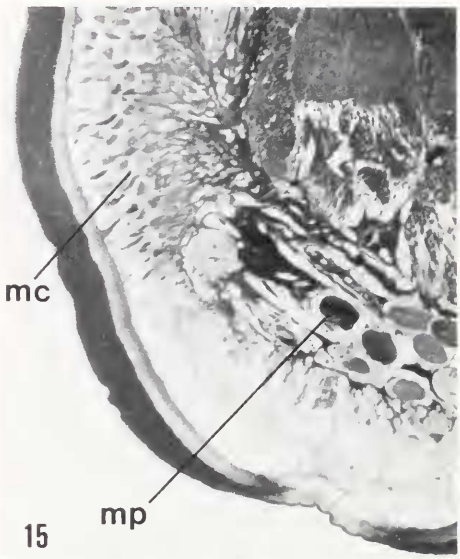
FIGURE 10. Illustrations of a pleopod from *P. macrodactylus*. Left; a right-side view, and right, a rear view. The pleopod consists of a coxapodite (cxp), a basipodite (bp), an endopodite (endo) and an exopodite (exo). A channel (ch) is found on the concave, posterior face of the basipodite. Eggs attach primarily to special setae (not shown) on the basipodites of brooding females.

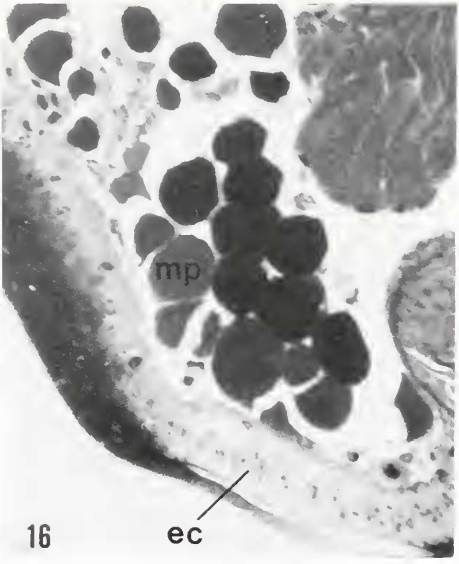
FIGURE 11. A scanning electron micrograph of the posterior face of a pleopod from a nonbrooding female, showing the central channel (ch) extending partway down the basipodite. 40×.

FIGURE 12. A cross-section through the proximal portion of the basipodite of a pleopod from an ovigerous (brooding) female shows the central channel (ch) and its intimate association with the external adhesive material (adh). Toluidine blue stain, 140×.

FIGURE 13. This cross-section through the basipodite of an ovigerous female shows an embryo (emb) lodged in the posterior concavity near the central channel (ch). The adhesive material (adh) encompasses the embryo and the pleopodal setae(s). Toluidine blue stain, 125×.







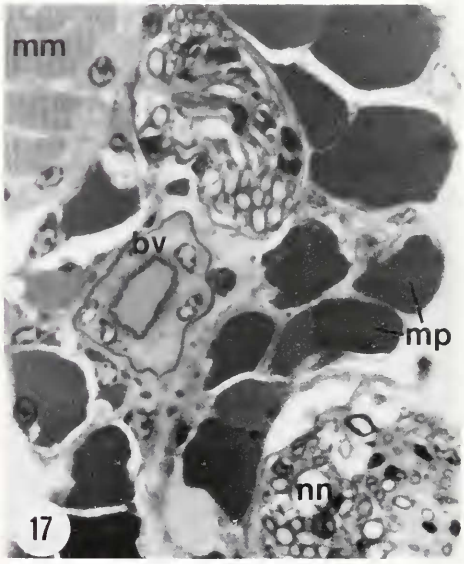


FIGURE 14. A cross-section through the coxapodite region shows the proximal portions of specialized epithelial cells, or mucilage cells (mc), that are believed to secrete material into mucilage packets (mp) for storage. Toluidine blue stain, 440×. (Inset shows the entire cross-section, 95×.)

FIGURE 15. The same pleopod (as shown in Fig. 14), sectioned a few microns distal, shows mucilage cells (mc) continuous with mucilage packets (mp), which contain a dark-staining material which is believed to be the precursor to adhesive material. Toluidine blue stain, 325×.

FIGURE 16. A cross-section of the same pleopod near the basipodite, shows the mucilage packets (mp) are not continuous with the more distal epithelial cells (ec) which elongate as the animal nears ecdysis. Toluidine blue stain, 300×.

FIGURE 17. In this cross-section of a different pleopod, mucilage packets (mp) are found in the center alongside muscle tissue (mm), blood vessels (bv) and nervous tissue (nn). Toluidine blue stain, 875×.

in that cement glands or ducts were not observed in *P. macrodactylus,* and secretion of adhesive material occurred before, rather than during, oviposition. It also differs from that proposed for *Carcinus* (Cheung, 1966) in that fertilization was not necessary for attachment and the outer layer was formed by material secreted from the female pleopods, not from individual eggs. Attachment in *Palaemonetes,* described by Burkenroad (1947) and Jefferies (1964) was probably the same as that described here for *P. macrodactylus,* but the adhesive material escaped detection because of its close conformity with the external surfaces of the pleopods.

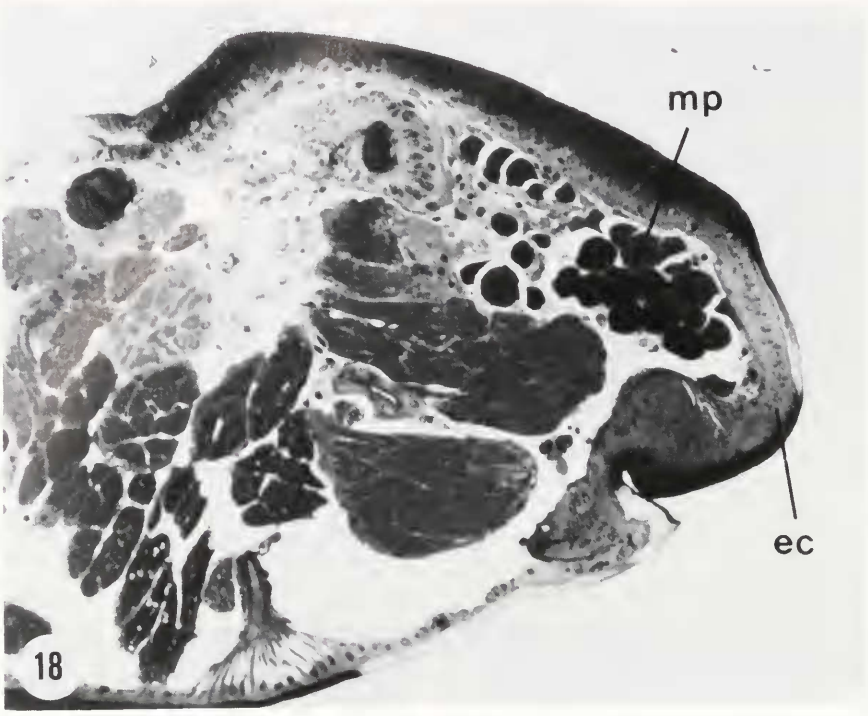
Eggs that were never attached (artificially produced by excision of the pleopods prior to extrusion) were not wrapped in this outer layer of material and, although they were slightly adherant, did not form connectants or strongly attach to each other. Clearly, the adhesive material had a major role in egg attachment. This externally applied layer also appeared to be responsible for protection of the inner coat (fertilization membrane for embryos, vitelline envelope for unfertilized eggs) and oolemma since unattached eggs deteriorated and became completely devoid of ooplasm within two days. A high number of bacteria inhabited the surfaces of unfertilized eggs without outer coats (Fisher, 1983) and may have obtained nutriment from escaping ooplasm or from the vitelline envelope.

It is not clear how the adhesive material is released, but it may be associated with the ecdysial process since it occurs at ecdysis rather than at oviposition. "Molting fluid" is believed to appear in the exuvial space (between the cuticle and the epidermal cells) in molting crustaceans (Kugler and Birkner, 1948; Needham, 1954) and in insects (Zacharuk, 1976) where resorptive enzymes in the fluid are secreted from the epidermal cells. Also in insects, Wigglesworth (1947) suggested that the outer layer of the exoskeleton is covered by a wax that is secreted by the epidermal cells immediately prior to ecdysis. In *P. macrodactylus,* the mucilage packets may secrete the stored mucilage into the exuvial space sometime between apolysis and ecdysis. After the exuvia is shed, the adhesive material coating the surfaces of the pleopods, as seen in Figures 13 and 19, is exposed.

Secretion can occur whether or not there are mature eggs in the ovary, implying that secretory activity is not regulated by an ovarian cycle. This is not without precedence; Stephens (1952) reached a similar conclusion concerning the activity of tegumental glands in the crayfish *Cambarus.* Cement glands in crayfish (Andrews, 1906; Stephens, 1952) and lobsters (Yonge, 1937) secrete at oviposition, a function not necessarily associated with molting events. In the Palaemonidae however, molting, mating and brooding are strictly sequential.

Although the importance of an externally applied outer investment coat has been stressed here, the role of the inner investment coat (fertilization membrane or vitelline envelope) should not be overlooked. Burkenroad's (1947) belief that the vitelline envelope had inherent fusibility properties is supported by the apparent coagulation on the surface of the vitelline envelope and the limited adherance of *P. macrodactylus* eggs teased from the ovary. Interaction between this coagulum and the externally applied adhesive material is very likely and may actually combine to form the outer investment coat. Concepts that considered pleopodal secretions as an "intensifier substance" (Burkenroad, 1947) or an investment-coat hardener (Cheung, 1966) may have been modified, but not disproven by the results of this study.

An acceptable general theory of decapod egg attachment is lacking, although several have been proposed. Yonge (1937) felt that all decapods would attach eggs in a manner similar to his description for *Homarus.* Hoglund (1943) also accepted this view. Cheung (1966) however, believed that his work with *Carcinus* established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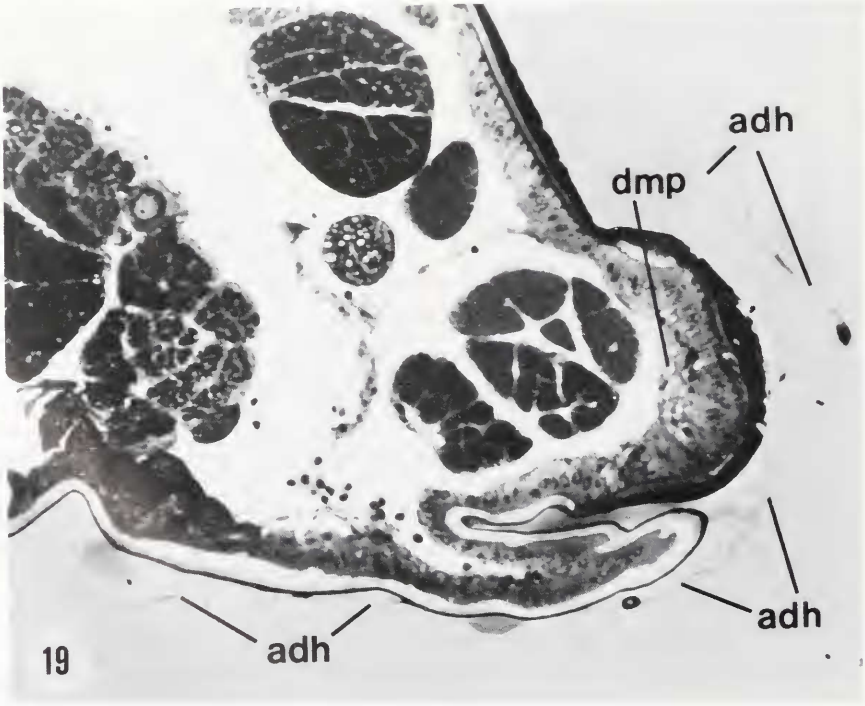
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the patterns followed by all brooding decapods. Under this assumption, Cheung (1966) incorrectly interchanged experimental results from *Carcinus* with histological results from *Astacus*. And although Cheung (1966) examined the macrurans *Nephrops* and *Homarus*, he used only a single preserved specimen of each and their differences from *Carcinus* were as prominent as their similarities. Burkenroad (1947) proposed a general theory that attempted to incorporate the discrepancies he had observed: he suggested that some eggs required an "intensifier substance" for attachment, whereas others were "self-fusible," resulting in both egg-to-egg and egg-to-seta attachment. He neglected the possibility, however, that egg-to-egg attachment might result from the hardening of an encompassing cement, such as the pouch of glaire observed by Andrews (1906), rather than activity by individual eggs.

The variations observed in different species prohibit description of a general theory or a typical system for decapod egg attachment. It may be possible, however, that there is similarity in their need for egg support during attachment. The carideans, as exemplified here, may have a fast-acting, externally applied adhesive material that attaches eggs held in place by long pleopods while the female stands upright. The macruran adhesive material may act more slowly (Andrews, 1906; Yonge, 1937) and, coupled with relatively shorter pleopods, may necessitate the unique and highly vulnerable inverted posture maintained during egg attachment. Brachyurans may have slowly attaching eggs (Cheung, 1966), apparently without benefit of an externally applied adhesive material, but attachment may be aided by their anatomical enclosure for the eggs (flexed abdomen) and, for some crabs, by their action of burying themselves in sand to support the eggs. These differences might reflect variations in the reproductive strategies of each section (infra-Order) of the Decapoda.

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FIGURE 18. A pleopod cross-section before ecdysis, shows the mucilage packets (mp) replete with dark-staining material. In this section, they are found close to the periphery of the pleopod, just inside the elongate epithelial cells (ec). Toluidine blue stain, 120×.

FIGURE 19. Cross-section of a pleopod immediately after ecdysis, shows the elongated epithelial cells are still present, but the mucilage packets are depleted (dmp) of their dark-staining material. The adhesive material (adh), which appears at this time on the external surfaces of the pleopod and pleopodal setae, is believed to be the dark-staining material secreted from the mucilage packets. Toluidine blue stain, 285×.

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